CLAIMS

1) Process for determining the activity *in vitro* of a substance using a functional test, characterized in that the variation of a known function corresponding either to one or several proteins produced *in vitro* in the presence and in the absence of said substance or to the substance in the presence or in the absence of proteins produced *in* vitro is detected and/or measured.

2) Process for determining the activity *in vitro* of a substance using a functional test according to claim 1, characterized in that it comprises the following steps:

   a) The preparation of at least one nucleic acid molecule comprising the gene(s) coding for one or several proteins and the control elements necessary for the transcription and the translation of said gene(s),
   b) The transcription of the nucleic acid molecule(s) prepared at step (a),
   c) The *in vitro* translation of the transcript(s) prepared at step (b),
   d) The detection and/or the measurement of the variation of a known function corresponding to the proteins produced at step (c) in the presence and in the absence of said substance or to the substance in the presence and in the absence of the proteins produced at step (c).

3) Process for determining the activity *in vitro* of a substance using a functional test according to one of claims 1 to 2, characterized in that the preparation of one or several nucleic acid molecules of step (a) consists of placing the gene(s) coding for said protein(s) under the control:

- For the transcription, of a 5' promoter and possibly of a 3' RNA polymerase terminator,
- For the translation, of a ribosome binding site upstream of said gene(s).

4) Process according to one of claims 1 to 3, characterized in that the functional test used corresponds to the detection and/or the measurement of a known function of the protein(s) produced at step (c).

5) Process of determining the activity *in vitro* of a substance using a functional test according to claim 4, characterized in that step (a) consists of the preparation of the nucleic acid molecule(s) by an amplification reaction of the gene(s) coding for said protein(s).

6) Process for determining the activity *in vitro* of a substance using a functional test according to claim 5, characterized in that step (a) consists of preparing the nucleic acid molecule(s) by an amplification reaction of the PCR or NASBA type of the gene(s) coding for said protein(s), with the aid of one or several pairs of primers, each one composed of:

- For the sense primer, some sequence hybridizing upstream of one or several nucleic acid molecules comprising the gene(s) coding for said protein(s), and of an RNA polymerase promoter and possibly a ribosome binding site, and
- For the antisense primer, some sequence hybridizing downstream of one or several nucleic acid molecules comprising the gene(s) coding for said protein(s), and possibly of an RNA polymerase terminator.

7) Process for determining the activity *in vitro* of a substance using a functional test according to one of claims 1 to 3, characterized

in that the functional test corresponds to the detection and/or to the measurement of a function of said substance.

8) Process for determining the activity *in vitro* of a substance using a functional test, according to claim 7, characterized in that step (a) consists (i) of preparing starting with a sample containing nucleic acids several nucleic acid molecules, each one comprising a nucleic acid fragment coming from said sample associated with a vector molecule, (ii) isolating each nucleic acid molecule composed of a nucleic acid fragment and of a vector molecule.

9) Process for determining the activity *in vitro* of a substance using a functional test according to claim 8, characterized in that said nucleic acid fragments preferably have a size of 1 to several dozens of kb, preferably from 1 to 40 kb and advantageously from 1 to 10 kb when the sample is of prokaryotic origin; and of the order of several dozens to several hundreds of kilobases in the case of a eukaryotic organism; in the particular case where eukaryotic cDNAs at step (a) are dealt with, the fragments will preferably have a size of 1 to 5 kb.

10) Process for determining the activity *in vitro* of a substance using a functional test, according to one of claims 8 or 9, characterized in that the vector molecule is composed of one or several polynucleotide sequences comprising at least one transcription promoter for step (b) and possibly an element facilitating the isolation of the nucleic acid fragment.

11) Process for determining the activity *in vitro* of a substance using a functional test, according to one of claims 8 to 10, characterized in that the vector molecule associated with each fragment of step (b) is advantageously a plasmidic vector preferably not permitting the expression of said fragment *in vivo*.

12) Process for determining the activity *in vitro* of a substance using a functional test, according to any one of the previous claims, characterized in that said function corresponds to a collection of target proteins of which the genes coding for these proteins are located on the same DNA fragment as in the case of an operon, or at different places of the DNA.

13) Process according to claim 12, characterized in that step (a) consists of preparing a nucleic acid molecule comprising the genes (the operon) coding for the proteins, 5' of the collection of said genes (from the operon) a DNA polymerase promoter, possibly 3' of the collection of said genes (from the operon) an RNA polymerase terminator, and for each of said genes its natural ribosome binding site.

14) Process according to claim 13, characterized in that the ribosome binding site of each one of the genes is its natural ribosome binding site, and that it is then preferred to use at step (c) a translation extract prepared starting from the organism that the target gene(s) come from or from a phylogenetically close organism.

15) Process according to claim 12, characterized in that step (a) consists of preparing one or several nucleic acid molecules comprising the genes coding for the proteins, 5' of each of said genes an RNA polymerase promoter and a ribosome binding site, and possibly 3' of each one of said genes an RNA polymerase terminator.

16) Process according to claim 15, characterized in that the ribosome binding site can be the natural site of each one of the genes or another ribosome binding site more adapted to the translation step (c).

17) Process for determining the activity *in vitro* of a substance using a functional test, according to one of the previous claims, characterized in that said proteins are variants of a protein or variants of a collection of proteins.

18) Process for determining the activity *in vitro* of a substance using a functional test, according to any one of the previous claims, characterized in that the detection and/or the measurement of the variation of function corresponding to the protein(s) produced at step (c) or to the substance is advantageously carried out at step (d) by a functional test using the presence, at one of steps (a), (b), (c) or (d), of one or several reporter molecules permitting detection and/or measurement of the activity of the protein(s) produced at step (c) or of the substance.

19) Process according to claim 18, characterized in that the reporter molecule is a molecule capable of directly or indirectly revealing the activity of one or several of said proteins or of said substance.

20) Process of determining the activity *in vitro* of a substance using a functional test, according to claim 19, characterized in that the reporter molecule is a protein that is produced during step (c) conjointly with said protein(s).

21) Process for determining the activity *in vitro* of a substance using a functional test, according to claim 20, characterized in that the gene coding for the reporter molecule is placed under the control of transcription and translation regulation sequences similar to those of the gene(s) coding for said protein(s), such that the reporter gene is co-expressed with said gene(s).

22) Process for determining the activity *in vitro* of a substance using a functional test, according to one of claims 1 to 17, characterized in that said protein or one of said proteins produced at step (c) is also a reporter molecule.

23) Process for determining the activity *in vitro* of a substance using a functional test, according to one of the previous claims, characterized in that said substance is introduced before, after and/or during the transcription and/or the translation of steps (b) and/or (c) and/or of detection and/or of measurement of the variation of at least one known function of step (d).

24) Process for determining the activity *in vitro* of a substance using a functional test, according to any one of the previous claims, characterized in that said substance is chosen among polynucleotides, peptides, proteins, ions, molecules or natural or synthetic chemical compositions, hormones, aromatic compounds, antibodies, antibody fragments, genes, cellular receptors, amino acids, glycopeptides, lipids, glycolipids, sugars, polysaccharides, antiviruses, inhibitors, stimulants, physico-chemical conditions, radiation, or thermal treatments.

25) Process for determining the activity *in vitro* of a substance using a functional test, according to any one of the previous claims, characterized in that after step (d), it is verified that said substance does not inhibit one of steps (a) to (c).

26) A kit for the implementation of a method according to any one of the previous claims, characterized in that it comprises: the means for revealing the function, an RNA polymerase, nucleotide sequences for the preparation of the nucleic acid molecules comprising the gene(s) permitting the expression of protein(s) corresponding to the detected and/or quantified function, the four triphosphate nucleotides, the mixtures

necessary for said preparation, to the transcription and to the translation, and possibly some controls.

27) A kit for the implementation of a method according to any one of claims 1 to 26, characterized in that it includes:

- Possibly the products necessary for the preparation of the nucleic acid molecules comprising the gene(s) permitting the expression of the protein(s) corresponding to the detected and/or quantified function,
- Any support such as microtitration plate or chip containing: the means for revealing a function, an RNA polymerase, the four triphosphate nucleotides, the transcription and translation mixtures, possibly substances, and possibly controls.

28) A support having a series of sites for the implementation of a method according to any one of claims 1 to 26, characterized in that each one of said sites permits the detection and/or the measurement of a variation of function.

29) Process for development of new functional tests characterized in that it comprises the following steps:

a) The preparation of at least one nucleic acid molecule comprising the gene(s) coding for one or several proteins and the control elements necessary for the transcription and the translation of said gene(s),

b) The transcription of the nucleic acid molecule(s) prepared at step (a),

c) The *in vitro* translation of the transcript(s) prepared at step (b),

d) The detection and/or the measurement of the variation of a known function corresponding to the proteins produced at step (c) in the presence and in the absence of one or several reporter molecule(s).